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Purification of a polyphenol oxidase isoform from potato (*Solanum tuberosum*) tubers

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Abstract

A different expression pattern of polyphenol oxidases has been observed during storage in cultivars of potato (*Solanum tuberosum* L.) featuring different length of dormancy: a short-dormant cultivar showed, at the end of the dormancy, both the highest polyphenol oxidase activity and the largest number of enzyme isoforms. An isoform of polyphenol oxidase isolated at the end of the physiological dormancy from a short-dormant cultivar has been purified to homogeneity by means of column chromatography on phenyl Sepharose and on Superdex 200. The purification factor has been determined equal to 88, and the molecular mass of the purified isoform has been estimated to be 69 and 340 kDa by SDS polyacrylamide gel electrophoresis and gel filtration on Superdex 200, respectively, indicating this PPO isoform as a multimer. The corresponding zymogram features a diffused single band at the cathodic region of the gel and the pI of this polyphenol oxidase has been calculated equal to 6.5.

Keywords: *Solanum tuberosum*; Solanaceae; Potato tuber; Enzyme expression; Purification; Polyphenol oxidase

1. Introduction

Polyphenol oxidase (EC 1.14.18.1 and EC 1.10.3.1; PPO) is a copper-containing enzyme, which catalyzes both the oxygen-dependent hydroxylation of monophenols to their corresponding *o*-diphenols and the oxidation of *o*-diphenols to their cognate *o*-quinones (Mayer and Harel, 1979). Quinones are electrophilic highly reactive molecules that can polymerise, leading to the formation of brown or black pigments (Mayer and Harel, 1991). These reactions have important implications in food managing and processing: upon browning, significant postharvest losses of several crops do occur (Vámos-Vigyázó, 1981). In potato, polyphenol oxidases are also related to blackspot, an internal damage of tuber observable during their storage and resulting from impact injuries (McGarry et al., 1996). During storage, PPO activity increases in the outer parts of potato tubers, and both the rate and the extent of the browning of tubers are cultivar-dependent (Vámos-Vigyázó, 1981). Despite its technological relevance, the molecular basis of this increase is not fully understood. Moreover, a close connection between the expression of polyphenol oxidases and potato dormancy has been suggested in several studies (for a review see Hemberg, 1985).

Thygesen et al. (1995) reported that polyphenol oxidase is present as a multigene family in potato and that each gene has a specific temporal and spatial pattern of expression: in particular, the transcription of all genes during potato development has been described, but no information is available about the regulation of polyphenol oxidase gene expression in mature tubers during dormancy. However, these authors suggest a transcriptional control of PPO expression in potato tissues: the increase of PPO activity during the storage of tubers is concomitant with the transcription of different PPO isoforms. This observation is in agreement with biochemical studies, which describe the purification to homogeneity of PPO from potato tubers (Partington and Bolwell, 1996; Pathak and Ghole, 1994), and report different molecular masses for the pure enzyme preparations. Accordingly, multiple PPO isoforms can be concomitantly present in potato tubers, due to the transcription of different genes or reflecting the presence

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in vivo of both the pre-enzyme (commonly observed for plastid proteins) and the corresponding mature form (Thygesen et al., 1995; Hunt et al., 1993). In potato, PPO does not feature latency (Vámos-Vigyázó, 1981): both the unprocessed and the mature form are active.

To understand the role of PPO in the modulation of the blackening response in plants and eventually in the control of dormancy in potato tubers, an immunological approach is necessary. Partington et al. (1999) have recently studied the location of potato PPO as affected by impact injury of tubers: these authors have performed immunogold location of PPO by using a polyclonal antibody specific for a particular isoform of potato tuber polyphenol oxidase. Moreover, Lanker et al. (1988) have suggested the use of polyclonal and monoclonal antibodies raised against broad bean polyphenol oxidase to detect PPO isoforms expressed in different species, but no information is available concerning the detection by this means of potato PPOs. Therefore, fast and easily reproducible protocols for the isolation of different PPO isoforms can be considered a critical step in studies aiming at the immunodetection of this enzyme.

The aim of this work was: (i) to study the expression of PPO in cultivars of potato in relation to the length of dormancy, (ii) to purify a non-processed polyphenol oxidase isoform from dormant potatoes.

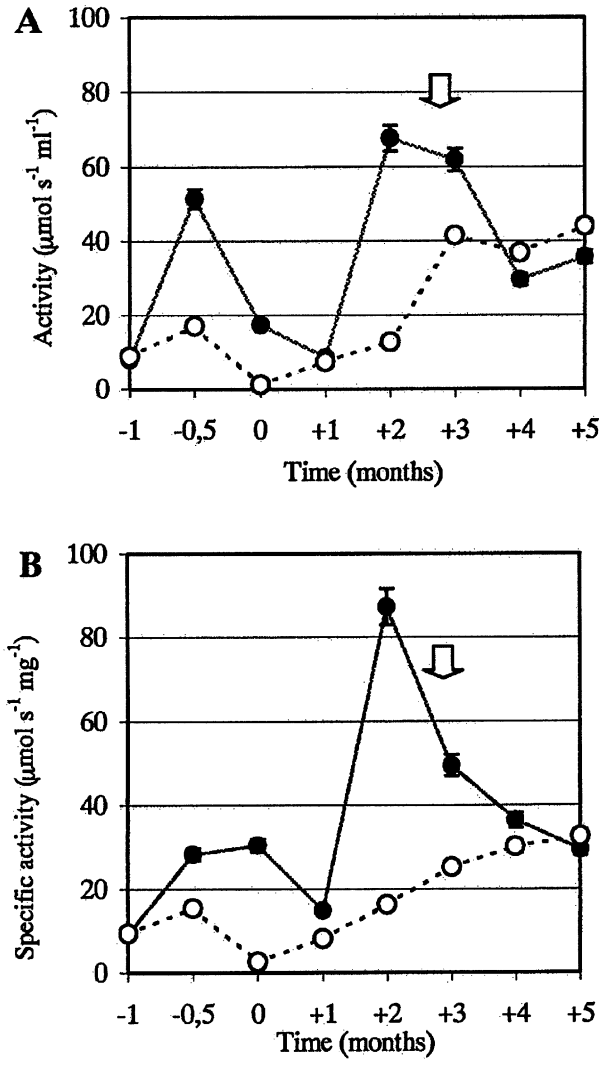

Fig. 1. PPO activity (A) and specific activity (B) in crude enzyme extracts of potato (closed circles cv Vivaldi, open circles cv Primura) peelings during tubers developing and storage. The arrows indicate the onset of sprouting of the tubers belonging to the cultivar Vivaldi.

2. Results and discussion

2.1. PPO activity during storage

Polyphenol oxidases expression has been studied in relation to the storage of two potato cultivars, namely Vivaldi and Primura, featuring length of dormancy equal to ca. 3 and 6 months, respectively. Both PPO activity (Fig. 1A) and PPO specific activity (Fig. 1B) in potato peeling crude extracts showed similar trends in both cultivars during the early dormancy: polyphenol oxidase activity was high before harvest of the tubers, and was found to decrease (in mature tubers) and to subsequently increase upon storage of the tubers until the start of the sprouting process; nevertheless, this rise of PPO activity was more pronounced in cv Vivaldi than in cv Primura. A decrease of either total and specific PPO activity was observed after sprouting in potato tubers of cv Vivaldi (see arrows in Fig. 1); tubers of cv Primura were continuously dormant during the time interval chosen for observation. These observations are in agreement with those reported by Hemberg (1985) describing high PPO activity associated with dormancy and a sharp decrease of activity occurring upon sprouting, and seem to support the hypothesis for a role of PPO in the physiology of dormant tubers.

A different pattern of PPO isoforms was found in tubers from the two different cultivars (Fig. 2A and B). During the time-course of storage, cv Vivaldi shows a higher number of PPO isoforms than cv Primura: in particular, cv Vivaldi features multiple cathodic forms, which could represent the aggregation of characteristic PPO isoforms as previously observed by Partington et al. (1999). The relative intensity of the bands forming the native profiles is clearly different in the two cultivars, and this difference could be correlated to the higher specific activity shown during storage by cv Vivaldi with respect to Primura. However, a direct comparison between native enzyme patterns and PPO activity does not consider the mechanisms of the physiological regulation of the enzyme activity. The diversity in PPO isoforms and the staining of native profiles are not punctually correlated to the correspondent levels of

PPO activity (cf. Figs. 1 and 2): this observation suggests the presence of activators/inhibitors in the enzyme crude extracts, which most likely does not affect the activity observed by means of native PAGE. Northern blotting of RNA isolated from peelings of tubers of cv Vivaldi collected at different stages during storage shows that the level of transcription of potato polyphenol oxidases mRNA (yielding a transcript of 2 kb) is relatively constant during dormancy (Fig. 3). The variation of the composition in PPO isoforms observed during tubers storage and shown in Fig. 2A could be the effect of the transcription of different RNAs (which are not discriminated by the probe used in this experiment) or the effect of a different enzyme processing, which could play a crucial role in the regulation of polyphenol oxidase activity in dormant potatoes. In conclusion, tubers from potato cultivars different in the length of dormancy feature different patterns of polyphenol oxidases. Considering each cultivar, the polyphenol oxidase activity most likely depends on the control of activators/inhibitors on different PPOs, resulting from a complex of post-transcriptional processes, which could be characteristic for every cultivar. Cv Vivaldi at the end of the dormancy features both the highest polyphenol oxidase activity and the largest number of enzyme isoforms: these tubers were chosen in order to purify an isoform of polyphenol oxidase.

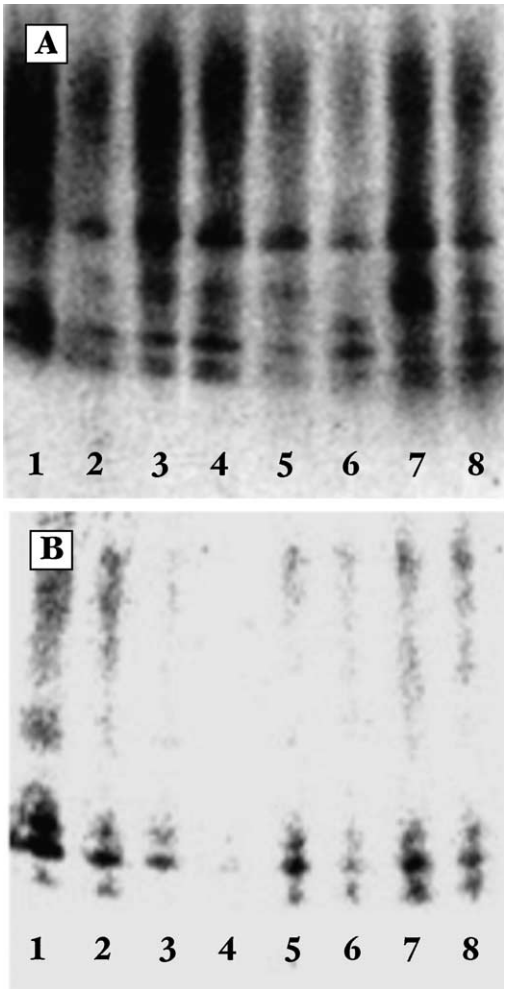

Fig. 2. Native PAGE patterns of PPOs from potato peelings of cv Vivaldi (A) and cv Primura (B) during the development and storage of tubers: results corresponding to 30 and 15 days before harvest (lanes 1 and 2, respectively); harvest (lane 3); 1, 2, 3, 4 and 5 months storage at 6–8 °C (lanes 4, 5, 6, 7, 8, respectively) are shown. Samples equalized to 0.1 mg of total protein either in A and B.

2.2. Purification of the enzyme

The purification of PPO described in the present work is based on phenyl Sepharose column chromatography. PPOs purified from different plant species feature variable extent of hydrophobicity (Yang et al., 2000, 2001; Fujita et al., 1995; Murao et al., 1993); in some purification approaches the hydrophobic interaction of the protein with phenyl Sepharose columns has been evaluated, equilibrating the sample in 1 M $(NH_4)_2SO_4$ (Söderhall, 1995; Robinson and Dry, 1992). Partington and Bolwell (1996) separated a potato polyphenol oxidase (appearing as a doublet in SDS–PAGE) from patatin, the major soluble glycoprotein of potato tuber and the strongest contaminant in purification, taking advantage of the higher affinity of patatin to an octyl Sepharose matrix. These authors reported the failure of an attempt to bind, following previously reported conditions, potato PPOs to a phenyl Sepharose column, even though this strategy was judged strategic as patatin binds weakly the aromatic phenyl group. The rationale of the purification scheme presented here is to identify conditions suitable to bind PPO to a phenyl Sepharose matrix differentially to patatin, then completing the purification by gel-filtration.

As a first step of the purification the removal of hydrophilic proteins from the crude extract was carried out using a batch of DEAE cellulose. This procedure

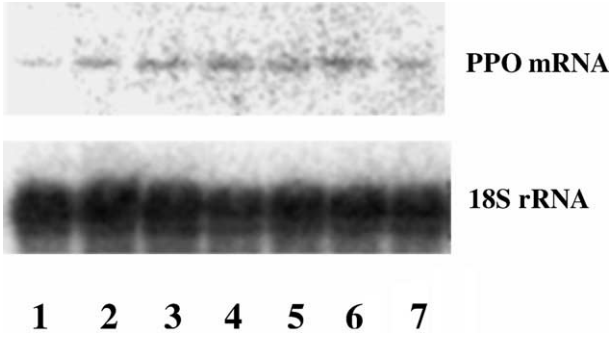

Fig. 3. PPO mRNA and 18S rRNA in potato (cv Vivaldi) peelings during tuber storage: results corresponding to harvest (lane 1); 15, 30, 45, 60, 75 and 90 days storage at 6–8 °C (lanes 2, 3, 4, 5, 6, 7 respectively) are shown. Samples equalized to 30 μg of total RNA.

(see Experimental) results in a partial loss of polyphenol oxidase activity, but ensured a higher homogeneity of the protein solution accordingly obtained; moreover, part of the phenols were adsorbed to the resin resulting in a consistent clearing of the crude extract. When the protein solution not previously adsorbed by DEAE cellulose was equilibrated to 1.5 M $(NH_4)_2SO_4$ and the corresponding supernatant was applied to a phenyl Sepharose column, no polyphenol oxidase activity was found in non-bound proteins. PPO was eluted from the phenyl Sepharose column within a broad peak at 0.2 M $(NH_4)_2SO_4$, and within two accompanying peaks at 0.5 and 0 M $(NH_4)_2SO_4$. Purification of the polyphenol oxidase was achieved by loading the major peak obtained with the phenyl Sepharose chromatography on a Superdex 200 column. A sharp peak of activity was observed in gel-filtration and the pure enzyme was found in the core fractions of this peak (Fig. 4).

Table 1 shows a summary of the purification procedure for the PPO: the protocol resulted in a 88-fold purification with a 0.5% recovery of activity from the fraction non-adsorbed to DEAE cellulose. A protein featuring molecular mass of about 40 kDa, which was the major one in crude extract and in both fractions of proteins adsorbed and non-adsorbed to DEAE cellulose (Fig. 5, lanes 1–3), presumably patatin, disappeared after phenyl Sepharose chromatography (Fig. 5, lane 4). A decrease of polyphenol oxidase activity was constantly observed in all steps of the purification procedure, as a consequence of the existence of different isoforms of the enzyme, which were discarded. Fig. 6

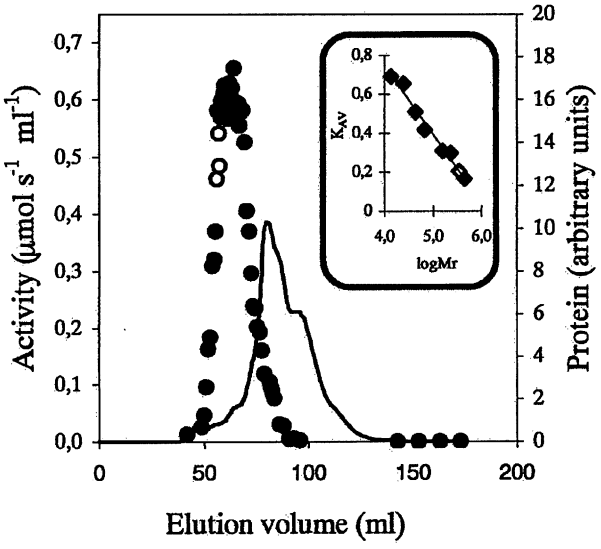

Fig. 4. Gel filtration chromatogram (Superdex 200) of potato PPO: ● PPO activity, ○ PPO activity in fractions containing the purified isoform; —protein. The inset shows the determination of the molecular mass of the native enzyme: ◆ correspond to molecular mass standards, ◇ corresponds to potato PPO).

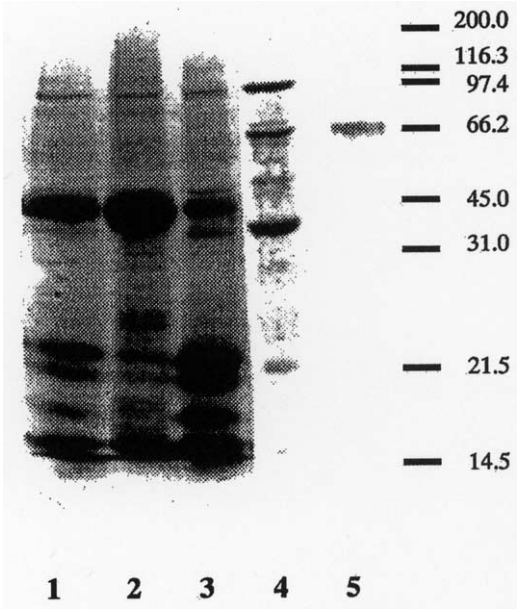

Fig. 5. SDS–PAGE of partially purified and purified PPO: crude extract (lane 1); protein solution obtained after adsorption on and desorption from DEAE cellulose (lane 2); protein non-adsorbed on DEAE cellulose (lane 3); active fractions eluted from phenyl Sepharose (lane 4); purified PPO (lane 5).

Table 1
Purification of polyphenol oxidase from potato (*Solanum tuberosum* cv Vivaldi) tubers

Purification step	Volume (ml)	Total activity ($\mu mol\ s^{-1}\ ml^{-1}$)	Total protein (mg)	Specific activity ($\mu mol\ s^{-1}\ ml^{-1}\ mg^{-1}_{protein}$)	Purification (fold)[a]	Recovery (%)
Crude extract	6750	212,946	3395.3	63		
DE52 batch	6750	12,054	317.3	38	1.0 (1.0)	100.0
Phenyl Sepharose	2.4	1196	7.8	153	4.0 (4.0)	9.9
Superdex 200	4.0	58	1.7E-02	3347	22.0 (88.1)	0.5

[a] In the calculation of the purification fold each step refers to the previous one; the purification fold indicated in parentheses is calculated referring to the DE52 batch step.

shows the composition of the isozyme profile at the main purification steps: different isoforms of the enzyme were observed even in correspondence of the advanced stages of the procedure.

2.3. Molecular mass determination

The molecular mass of the native enzyme was calculated to be approximately equal to 340 kDa when estimated by means of Superdex 200 gel filtration (Fig. 4). On SDS-PAGE, under completely denaturing conditions, the purified PPO features a single band corresponding to a molecular mass of 69 kDa (Fig. 5, lane 5). These results indicate that this PPO isoform is a multimer. Moreover, the enzyme features a marked trend toward aggregation into high molecular mass complexes: a diluted protein solution loaded on Superdex 200 resulted in a single peak, with high PPO activity, eluted in the void volume. The molecular mass of the monomer is in agreement with that of unprocessed PPO predicted by Thygesen et al. (1995) for mature potato tuber and could be a PPO isoform provided of the transit peptide. Similar results were shown by Partington and Bolwell (1996) who reported a doublet of polyphenol oxidase, purified from potato tubers and featuring molecular masses of 69 and 60 kDa; a similar N-terminal for the two forms, when sequenced, has been found. The antibody against the 69 kDa isoform, obtained by the same authors after further purification, has been used to perform Western blots of crude protein extracts from tubers (Partington et al., 1999): the antiserum recognized 69 kDa PPOs and its multimers, but no bands of 60 kDa were observed in blots. This observation can be related to that of Hunt et al. (1993), who, in immunoblots of potato tubers extracts stained with an antibody against *S. berthaultii* 59 kDa PPO, found selectively only 60 kDa PPO isoforms, suggesting that the two 60 and 69 kDa PPO isozymes contain different antigen determinants that limit antibody cross-reactivity. According to these observations, the polyphenol oxidase isoform purified in the present paper could be used to prepare antibodies probing 69 kDa PPO in potato: in fact, the protocol described is fast, reproducible and allows the direct purification of a 69 kDa PPO isoform from potato tubers. The only PPO isoform, other than that described by Partington and Bolwell (1996), purified from potato tubers to homogeneity was reported by Pathak and Ghole (1994); nevertheless, this isoform showed a molecular mass of 60 kDa and was supposed to be a mature processed isoenzyme. This leads to the conclusion that the protocol presented in the present paper is the only one available describing the purification to homogeneity of a 69 kDa PPO isoform from potato tubers.

2.4. Native PAGE and isoelectric focusing of the enzyme

Analysis of purified PPO by native PAGE exhibited a single diffused band of activity at the cathodic region of the gel (Fig. 6, lane 5). The isoelectric point of the purified enzyme is about 6.5 as measured by isoelectric focusing (Fig. 7). These observations are consistent with

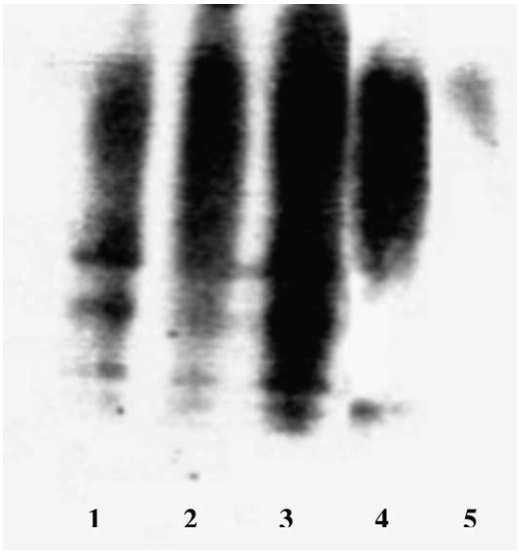

Fig. 6. Native PAGE stained for PPO activity of partially purified and purified enzyme: crude extract (lane 1); protein pooled after adsorption on DEAE cellulose (lane 2); protein non-adsorbed on DEAE cellulose (lane 3); active fractions eluted from phenyl Sepharose (lane 4); purified PPO (lane 5).

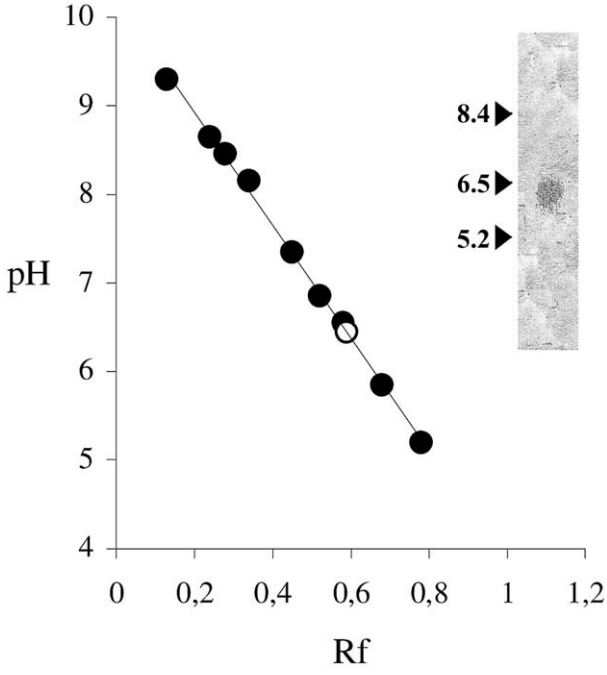

Fig. 7. Determination of the pI of the enzyme. The inset shows the isoelectric focusing of purified PPO stained for enzyme activity.

both the hydrophobic and the multimeric properties of the purified enzyme. In particular, the pI value of this enzyme is higher than those generally reported for PPOs, although already previously found for potato polyphenol oxidases: in enzyme preparations from potato tubers, Thomas et al. (1978) separated eleven PPO isoforms by thin-layer isoelectric focusing on Sephadex G75, founding pIs in the range of 4.8–6.8.

3. Experimental

3.1. Materials

Potato (*Solanum tuberosum* L. cvs Vivaldi and Primura) tubers were obtained from an experimental trial and stored at 6-8 °C. DEAE-cellulose (DE52) was obtained from Whatman International Ltd. (Maidstone, Kent, UK); phenyl Sepharose fast flow and Superdex 200, as well as Phast Gels IEF 3-9 and pI Standards 3-9 (Isoelectric Focusing Calibration Kit) were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). The Bradford protein assay reagent, bovine serum albumin and reagents for electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA). All other reagents were of analytical grade.

3.2. Enzyme assay

PPO activity was determined spectrophotometrically at 20 °C in triplicate. The reaction mixture (1 ml) consisted of 0.1 M potassium phosphate buffer, pH 5.7, 20 mM 4-methylcatechol and 5–100 μl of the sample. The increase in absorbance was followed in a 1 cm light path cuvette at 400 nm, in a final volume of 1 ml: PPO activity was expressed as millimoles of the corresponding *o*-quinone produced per second (mmol s^{-1}) considering a molar extinction coefficient of 1400 M^{-1} cm^{-1} (Waite, 1976).

3.3. Protein determination

Protein concentration was determined according to the method of Bradford (1976), using bovine serum albumin as standard. To monitor proteins in column eluates the absorbance at 280 nm was determined.

3.4. Enzyme extraction

Peelings from potato tubers were homogenized at 4 °C for 2 min with 3 volumes of cold acetone, and the residue obtained by filtration was homogenized again with cold acetone (−20 °C, 1:2, w/v). The precipitate was collected by filtration and was spread to dry it at 20 °C overnight. Proteins were extracted at 4 °C, by suspending portions of dried acetone powders in 1:10 (w/v) 10 mM potassium phosphate buffer, pH 5.7, containing 5 mM sodium ascorbate and 1 mM phenylmethylsulfonyl fluoride. After being shaken for 1 h, the suspension (crude extract of the enzyme) was centrifuged at 10,000 *g* for 10 min, at 4 °C.

3.5. Native polyacrylamide gel electrophoresis (native PAGE)

Native electrophoresis was performed with PPO crude extracts according to the method of Laemmli (1970) without SDS, using a Miniprotean II dual slab cell unit (Bio-Rad) and 10% polyacrylamide gels. Gels were stained for PPO activity in 0.1 M potassium phosphate buffer (pH 5.7) containing catechol and *p*-phenylenediamine (Yu et al., 1992) at the concentrations of 10 mM and 0.05% (w/v), respectively.

3.6. Enzyme purification

A crude extract from 5.4 kg peelings of tubers cv Vivaldi was obtained as described above and equilibrated at pH 7.6 with 50 mM Tris. After stirring for 1 h with DEAE-cellulose, the solution was collected after filtration and concentrated using a stirred ultrafiltration cell (Amicon). After addition of $(NH_4)_2SO_4$ to 1.5 M the centrifuged solution was loaded onto a phenyl Sepharose column (1.6 × 30 cm), previously equilibrated with 50 mM Tris (pH 7.6), 1.5 M $(NH_4)_2SO_4$. Bound proteins were eluted using a reverse gradient (0.5–0 M) of $(NH_4)_2SO_4$. Active fractions were pooled, dialysed against 10 mM Tris pH 8.0, and then concentrated using Microcon YM-10 ultrafiltration cells (Amicon). The most active fractions were equilibrated to 0.150 M NaCl, centrifuged at 10,000 *g* for 10 min at 4 °C and loaded onto a 1.6 × 60 cm Superdex 200 gel permeation column equilibrated with 10 mM Tris (pH 8.0), 0.150 M NaCl. Active fractions were pooled and concentrated using Microcon YM-10 (Amicon). All chromatographic steps were carried out at 4 °C.

3.7. Molecular mass determination

The molecular mass of the purified enzyme was determined by gel permeation and SDS–PAGE. Gel permeation was done as described above using ribonuclease A, chymotrypsinogen A, ovalbumin, albumin, aldolase, catalase and ferritin (M_r equal to 13,700, 25,000, 43,000, 67,000, 158,000, 232,000, 440,000 Da, respectively) as marker proteins. SDS–PAGE was performed according to the method of Laemmli (1970) using 12.5% polyacrylamide gels. Protein bands were stained with Coomassie brilliant blue R-250 and lysozyme, trypsin inhibitor, carbonic anhydrase, ovalbumin, serum albumin, phosphorylase b, β-galactosidase and myosin (M_r equal to 14,400, 21,500, 31,000, 45,000,

66,200, 97,400, 116,250, 200,000 Da, respectively) were used as markers of molecular mass under denaturing conditions.

3.8. Isoelectric focusing (IEF)

Electrophoresis was performed using a Phast System apparatus (Amersham-Pharmacia Biotech) and ready gels Phast Gel IEF 3-9; standards (3-9) of the Isoelectric Focusing Calibration Kit were used as markers of pI. Gels were stained for PPO activity in 0.1 M potassium phosphate buffer (pH 5.7) containing 10 mM catechol and 0.05% (w/v) *p*-phenylenediamine. Protein bands were stained with Coomassie brilliant blue R-250.

3.9. RNA analysis

Plant tissues were frozen in liquid nitrogen and ground to a fine powder. RNA was extracted and isolated after precipitation in 2 M LiCl and, subsequently, in ethanol, as described by Nagy et al. (1987). Total RNA was fractionated through MOPS-formaldehyde 1% gels (loading based on an equal amount of RNA) and transferred to nylon membranes (Hybond N+, Amersham Pharmacia Biotech) according to Sambrook et al. (1989). The filters were hybridised with a 1×10^5 cpm ml^{-1} ^{32}P-labeled double stranded DNA probe, using the protocol described by Mittler and Zilinskas (1994). To obtain this probe a *Cla*I-*Nco*I fragment of the ppoA gene from *Lycopersicon esculentum* subcloned in pSP64 (Koussevitzky et al., 1998) was used as template to label the complementary strand. The radioactive strand was synthesized by DNA polymerase Klenow fragment in the presence of ^{32}P-labeled dCTP and a mixture of unlabeled dGTP, dATP, dTTP nucleotides. By the same procedure a 562-nucleotide ^{32}P-labeled double stranded probe was synthesized using as template a *Eco*RI fragment of the pea 18S ribosomal RNA gene described by Mittler and Zilinskas (1994). After hybridisation, membranes were washed under the following conditions: two 10 min washes in 2× SSC and 0.1% SDS at 37 °C and two 30 min washes in 0.1× SSC and 0.1% SDS at 55 °C (1× SSC = 150 mM NaCl and 15 mM Na citrate, pH 7.0).

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References

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein–dye binding. Analytical Biochemistry 72, 248–254.

Fujita, S., bin Saari, N., Maegawa, M., Tetsuka, T., Hayashi, N., Tono, T., 1995. Purification and properties of a polyphenol oxidase from cabbage (*Brassica oleracea* L.). Journal of Agricultural and Food Chemistry 43, 1138–1142.

Hemberg, T., 1985. Potato rest. In: Li, P.H. (Ed.), Potato Physiology. Academic Press, London, pp. 353–388.

Hunt, M.D., Eannetta, N.T., Yu, H., Newman, S.M., Steffens, J.C., 1993. cDNA cloning and expression of potato polyphenol oxidase. Plant Molecular Biology 21, 59–68.

Koussevitzky, S., Ne'eman, E., Sommer, A., Steffens, J.C., Harel, E., 1998. Purification and properties of a novel chloroplast stromal peptidase. Journal Biological Chemistry 273, 27064–27069.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lanker, T., Flurkey, W.H., Hughes, J.P., 1988. Cross-reactivity of polyclonal and monoclonal antibodies to polyphenoloxidase in higher plants. Phytochemistry 27, 3731–3734.

Mayer, A.M., Harel, E., 1979. Polyphenol oxidase in plants. Phytochemistry 18, 193–215.

Mayer, A.M., Harel, E., 1991. Phenoloxidases and their significance in fruit and vegetables. In: Fox, P.F. (Ed.), Food Enzymology, Vol. 1. Elsevier, New York, pp. 373–398.

McGarry, A., Hole, C.C., Drew, R.L.K., Parson, N., 1996. Internal damage in potato tubers: a critical review. Postharvest Biology and Technology 8, 239–258.

Mittler, R., Zilinskas, B., 1994. Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. Plant Journal 5, 397–406.

Murao, S., Oyama, H., Nomura, Y., Tono, T., Shin, T., 1993. Purification and characterization of *Arctium lappa* L. (edible burdock) polyphenol oxidase. Bioscience Biotechnology and Biochemistry 57, 177–180.

Nagy, F., Boutry, M., Hsu, M.Y., Wong, M., Chua, N.H., 1987. The 5′-proximal region of the wheat Cab-1 gene contains a 268-bp enhancer-like sequence for phytochrome response. EMBO Journal 6, 2537–2542.

Partington, J.C., Bolwell, G.P., 1996. Purification of a polyphenol oxidase free of the storage protein patatin from potato tuber. Phytochemistry 42, 1499–1502.

Partington, J.C., Smith, C., Bolwell, G.P., 1999. Changes in the location of polyphenol oxidase in potato (*Solanum tuberosum*) tuber during cell death in response to impact injury: comparison with wound tissue. Planta 207, 449–460.

Pathak, S.U., Ghole, V.S., 1994. Affinity purification and properties of polyphenoloxidase from *Solanum tuberosum*. Phytochemistry 36, 1165–1167.

Robinson, S.P., Dry, I.B., 1992. Broad bean leaf polyphenol oxidase is a 60-kilodalton protein susceptible to proteolytic cleavage. Plant Physiology 99, 317–323.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning—A Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. pp. 7.43–7.48.

Söderhall, I., 1995. Properties of carrot polyphenoloxidase. Phytochemistry 39, 33–38.

Thomas, P., Delincée, H., Diehl, J.F., 1978. Thin-layer isoelectric focusing of polyphenoloxidase on Sephadex and its detection by the print technique. Analytical Biochemistry 88, 138–148.

Thygesen, P.W., Dry, I.B., Robinson, S.P., 1995. Polyphenol oxidase

in potato. A multigene family that exhibits differential expression patterns. Plant Physiology 109, 525–531.

Vámos-Vigyázó, L., 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. CRC Critical Reviews in Food Science and Nutrition 15, 49–127.

Waite, J.H., 1976. Calculating extinction coefficients for enzymatically produced o-quinones. Analytical Biochemistry 75, 211–218.

Yang, C.P., Fujita, S., Ashrafuzzaman, M.D., Nakamura, N., Hayashi, N., 2000. Purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.) pulp. Journal of Agricultural and Food Chemistry 48, 2732–2735.

Yang, C.P., Fujita, S., Kohno, K., Kusubayashi, A., Ashrafuzzaman, M.D., Hayashi, N., 2001. Partial purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.) peel. Journal of Agricultural and Food Chemistry 49, 1446–1449.

Yu, H., Kowalski, S.P., Steffens, J.C., 1992. Comparison of polyphenol oxidase expression in glandular trichomes of *Solanum* and *Lycopersicon* species. Plant Physiology 100, 1885–1890.